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Lab resource: stem cell line

Episomal plasmid-based generation of an iPSC line from a 79-year-old individual carrying the *APOE4/4* genotype: i11001

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ABSTRACT

In this study, lymphoblastoid cells derived from a 79-year old individual with a history of progressive presenile dementia, were used to generate iPS cells, employing episomal plasmids expressing OCT4, SOX2, KLF4, LIN28, L-MYC and a p53 shRNA. The individual was homozygous for the *APOE4* allele. The resulting iPS cells had a normal karyotype, retained the *APOE4/4* genotype, expressed pluripotency markers, were free of genomically integrated plasmids, and could be differentiated into cell type representatives from the three germ layers *in vitro*.

Resource table.

Name of Stem Cell line	i11001
Institution	Philipps University Marburg
Person who created resource	Shadaan Zulfiqar
Contact person and email	Katja Nieweg, nieweg@staff.uni-marburg.de
Date archived/stock date	Nov 24, 2015
Origin	EBV transformed human B lymphocytes (AG11001 from NIA Aging Cell Culture Repository)
Type of resource	Biological reagent: induced pluripotent stem cell, derived from human lymphoblastoid cells
Sub-type	Cell line
Key transcription factors	OCT4, SOX2, LIN28, L-MYC pCXLE-hOCT3/4-shp53-F (ID 27077) pCSLE-hSK (ID 27078) pCXLE-hUL (ID 27080)
Authentication	Identity and purity of cell line confirmed (as shown in Figure1)
Link to related literature	http://www.ncbi.nlm.nih.gov/pubmed/21556001
Information in public databases	Not available

Resource details

The generation of human IPSC line i11001 was carried out using E8 based media and feeder-free conditions. EBV-transformed B-lymphocyte cells from a 79-year old Alzheimer's Disease (AD) patient, generated by Coriell Institute For Medical Research, were employed. The patient had a history of progressive presenile dementia, with a parent and three siblings of the patient also being affected (iPSC line i10984). The patient was homozygous for the *APOE4* allele. This was confirmed by a PCR-RFLP assay (Hixson and Vernier, 1990) carried out with the lymphoblastoid cell line (Fig. 1A).

The lymphoblastoid cells (LCs) were electroporated with OriP/EBNA-1 based episomal plasmids expressing reprogramming factors OCT4, SOX2, KLF-4, L-MYC, LIN28 and a p53 shRNA (Okita et al., 2011). The *APOE4/4* genotype of the generated iPS line was confirmed by sequencing (Fig. 1A). After several passages, a PCR for episomal vector backbone confirmed that exogenous episomal factors were not being expressed anymore (Fig. 1A).

i11001 iPSC colonies displayed a typical ES-like colony morphology (Fig. 1B). Immunocyto-chemical analysis revealed expression of transcription factors OCT4 and SOX2, and surface markers SSEA4 and TRA-1-81, characteristic of pluripotent stem cells (Fig. 1B). The iPS cells exhibited a normal karyotype (46, XX) by G-Banding analysis (Fig. 1B). The cells also demonstrated differentiation capacity to three germ layers using an embryoid body-based directed differentiation protocol, followed by immunostaining with markers corresponding to the three germ layers i.e. ectoderm (PAX6), mesoderm (FOXC1) and endoderm (GATA4) (Fig. 1C).

Materials and methods

Cell culture

The LC line was cultured in RPMI-1640 Medium (Gibco™) supplemented with 15% FBS (MP Biomedicals), 1% Penicillin/Streptomycin (Invitrogen™) and 1% GlutaMAX (Invitrogen™) at 37 °C and 5% CO_2.

Derivation of the iPS cell line and expansion

LCs were reprogrammed by electroporating 200,000 cells with OriP/EBNA-1-based episomal plasmids (the plasmids were a gift from Shinya

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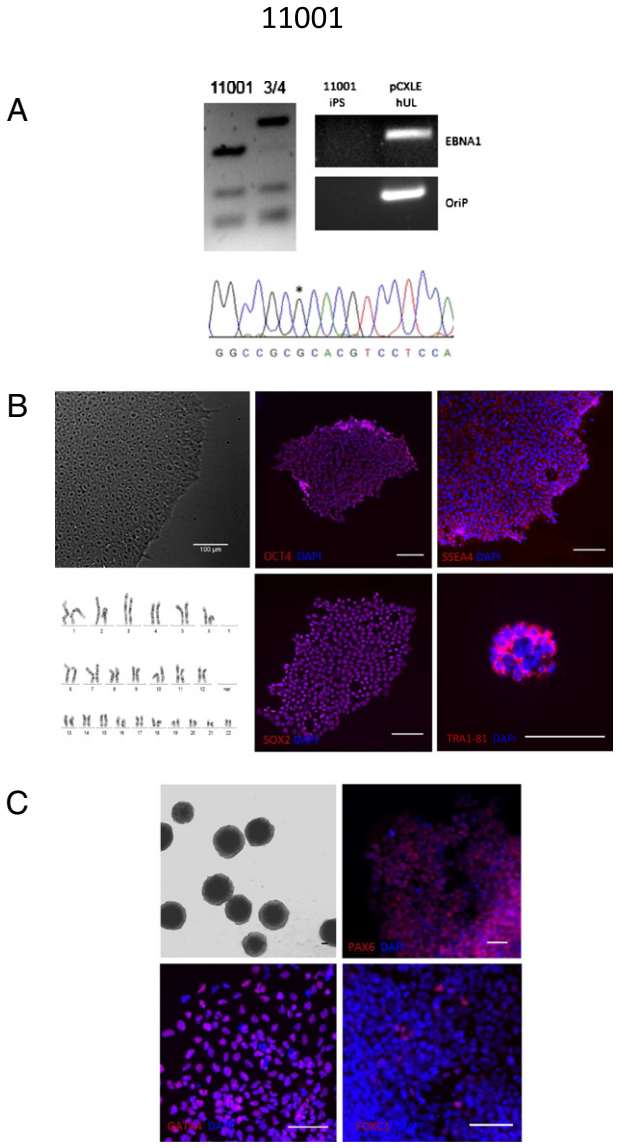

Fig. 1. Generation and characterization of i11001 line. (A) Confirmation of *APOE4/4* status of lymphoblastoid (source) line and i11001 iPS line by PCR-RFLP assay and Sanger sequencing, respectively. (Sequencing trace is reverse complement of sense strand). Product of OriP and EBNA PCR run with a positive control, shows episomal plasmids have been lost by the iPSCs. (B) i11001 cellular morphology resembles ES-like cell morphology. Immunofluorescence staining for pluripotency markers OCT4, SOX2, SSEA4, TR-A1-81 is positive. Karyotype is normal (46, XX). Scale bar: 100 μm (C) EB formation was induced, followed by directed differentiation to, and immunofluorescence staining for markers of, the three germ layers: Ectoderm: PAX6, Mesoderm: FOXC1, Endoderm: GATA4. Scale bar: 100 μm.

Yamanaka) encoding OCT3/4, SOX2, KLF4, L-MYC, LIN28, and shRNA of p53 (Okita et al., 2011) using a Neon transfection system (Invitrogen™). 1 μg of each plasmid and 10 ul tip of the Neon microporator kit were used for 2 pulses of 30 ms each at 1100 V. The cells were plated on Matrigel (Corning®) coated plates, in E8 medium without TGF-β, supplemented with 100 μM sodium butyrate. After 15–17 days, colonies started to appear and when they reached a sufficient size, were manually picked and plated onto Matrigel (Corning®) coated plates, in complete E8 medium. Cells were passaged by exposing to DPBS (without Ca^{++} & Mg^{++}) for a few minutes, then scraping the cell aggregates with a scraper, followed by mechanical trituration and subculturing. Cells were frozen using CryoStor® (Sigma-Aldrich).

Polymerase chain reaction

PCR for OriP and EBNA-1 was carried out using primers as described in Cai et al. (2015). PCR reaction was performed using Kapa HiFi Polymerase (Kapa Biosystems) with cycling conditions: 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min for 35 cycles. Episomal vector pCXLE-hUL was used as a positive control. The products were run on a 2% agarose gel.

Genotyping of APOE sequence

PCR of APOE exon 4 was carried out using the primers:

Forward: 5′CAGTCTCTCACACTCGTCCTGGC3′; Reverse: 5′CTGCTCCT TCACCTCGTCCA3′ PCR reaction was performed using Kapa HiFi Polymerase (Kapa Biosystems) with cycling conditions: 94 °C for 30 s, 61 °C for 30 s, 72 °C for 1 min for 30 cycles.

Sanger sequencing was then performed on the PCR product using the reverse primer by Eurofins Genomics.

APOE genotyping by RFLP assay

Genotyping for APOE status of the LCs was carried out by PCR amplification followed by *HhaI* digestion as described in Hixson and Vernier (1990). An *APOE3/4* line DNA was run as a reference.

Karyotyping

Karyotyping of the iPS cells was performed as described in Campos et al. (2009), followed by G-Banding. Metaphases were imaged using a Zeiss Axioskop, 60×, and analysed using Ikaros software (Metasystems). At least 30 metaphases were counted.

Embryoid body (EB) formation and directed differentiation

For EB formation, iPSC colonies were first dissociated to single cells using Accutase® (Sigma-Aldrich), counted and cultured, hanging as

Table 1
Antibodies used for immunocytochemistry.

	Antibody	Dilution	Company (Cat #)
Pluripotency Markers	Rabbit anti-OCT4	1:300	Cell Signalling Technology #9656
	Rabbit anti-SOX2	1:300	Cell Signalling Technology #9656
	Mouse anti-SSEA4	1:300	Cell Signalling Technology #9656
	Mouse anti-TRA-1-81	1:300	Cell Signalling Technology #9656
Differentiation Markers	Rabbit anti-FOXC1	1:100	Bethyl Laboratories Inc. #A303-520 A
	Rabbit anti-GATA4	1:200	Bethyl Laboratories Inc. #A303-503 A
	Mouse anti-PAX6	1:100	Developmental Studies Hybridoma Bank #AB 528427
Secondary antibodies	CF488A Goat Anti-Mouse IgG (H + L)	1:1000	Biotium Inc. #20010
	CF488A Goat Anti-Rabbit IgG (H + L)	1:1000	Biotium Inc. #20019

single drops of 2000 cells/20 ul on lids of petri dishes in E8 medium supplemented with 4 mg/ml polyvinyl alcohol.

For differentiation into germ layer precursors, small molecules were used as described in Frank et al. (2012). Briefly, for differentiation to neuroectoderm, 5 days of treatment with FTDA medium supplemented with 0.5 mM PD0325901, 15 mM SB431542 and 0.5 mM Dorsomorphin was applied. For mesodermal differentiation, treatment for 2 days with 10 ng/ml FGF2, 5 ng/ml Activin A, 10 ng/ml BMP4 (R&D), and 5 mM CHIR99021 was carried out. Endodermal differentiation was induced by treatment with 10 ng/ml FGF2, 100 ng/ml Activin A, 10 ng/ml BMP4, and 5 mM CHIR99021 for 1d, and then with 100 ng/ml Activin A for 3 days. Following this treatment, plated cells were immunostained.

Immuno cytochemistry

Expression of pluripotency and germ layer markers was verified using immunocytochemical staining on cells plated on coverslips. Cells were fixed in 4% PFA (15 min, room temperature (RT)), permeabilized (with the exception of surface markers) with 0.1 M Glycine and 0.25% triton in PBS (10 min, RT). This was followed by blocking in 30% normal goat serum (1 h, RT) and incubation with primary (overnight, 4 °C) and secondary (1 h, RT) antibodies (see Table 1) in 2% BSA/PBS. Nuclei were stained using 1 μg/ml DAPI (1 h, RT). Cells were imaged using a Leica DMI6000 B.

Acknowledgements

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